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**(54) Electrically promoting the bioreaction of microorganisms**

**(57) The bioreaction caused by a microorganism in a liquid culture can be enhanced by treating said culture with an electric stimulus.**

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Fig. 1

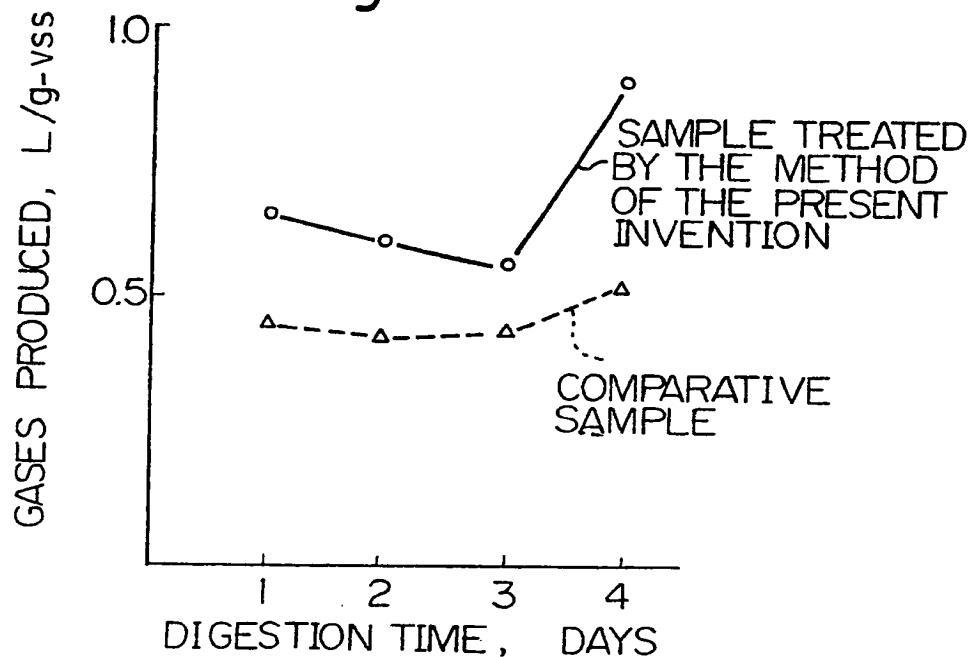
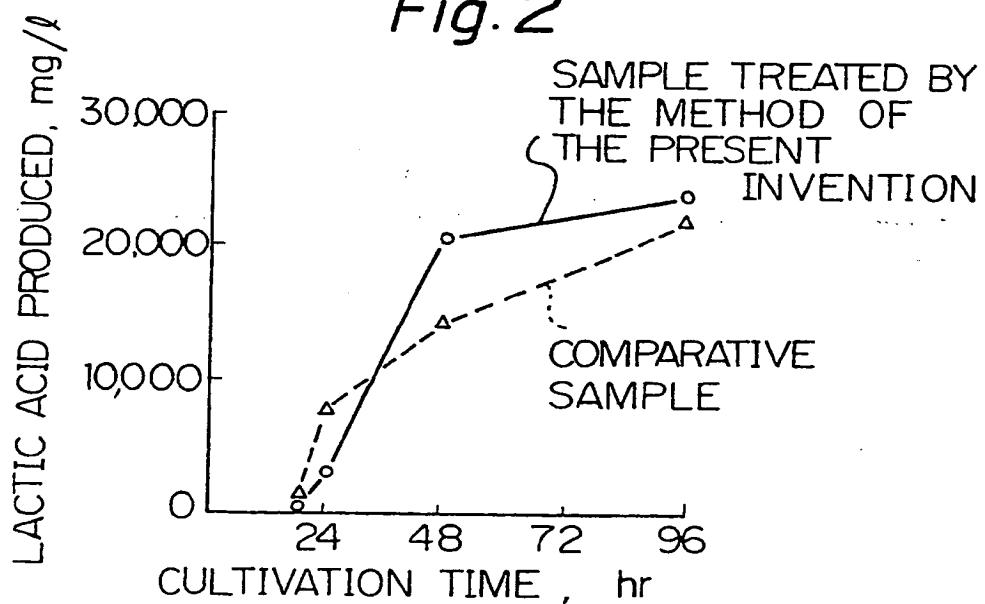


Fig. 2



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Fig. 3

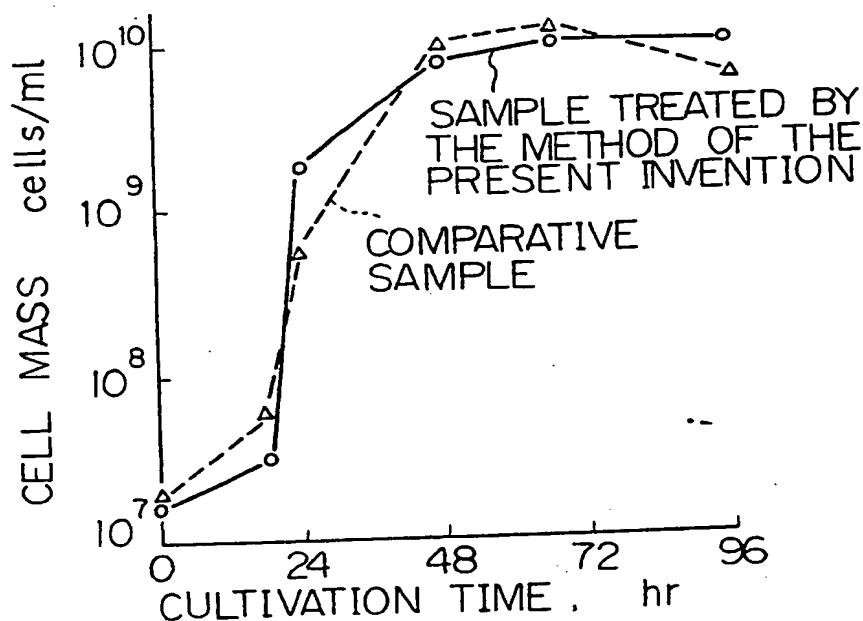
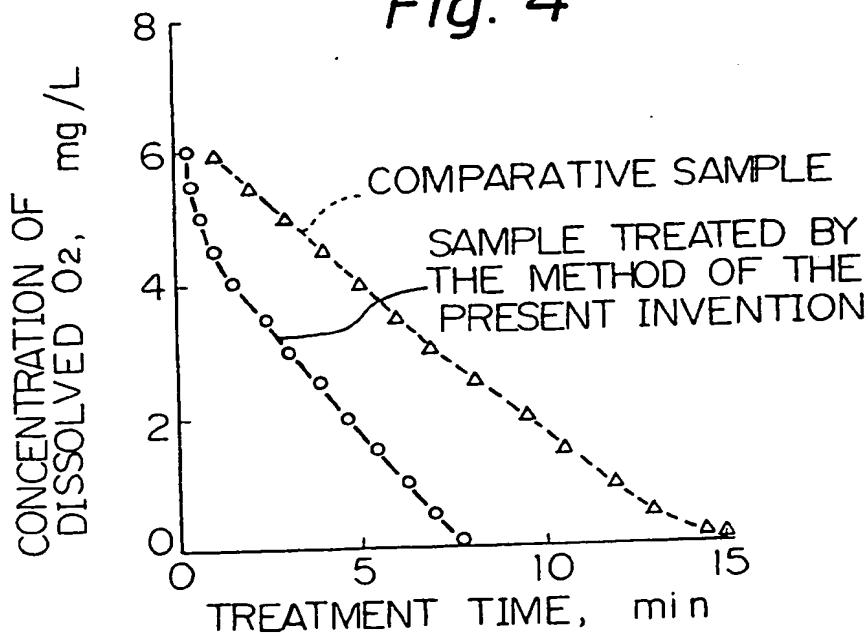


Fig. 4



**SPECIFICATION****Method of promoting the bioreaction of microorganisms**

5 The present invention relates to a method of promoting the activity of microorganisms in a liquid culture by applying an electrical stimulus to them.

10 Bioreaction, or the reactions caused by microorganisms, is currently used in the fermentative production of *miso* and *shoyu*, digestion of sludge, and the activated sludge treatment of sewage. Unlike chemical reactions, bioreaction requires no high pressure and temperature to carry on. Furthermore, bioreaction consumes less energy than chemical reactions. However, the rate of bioreaction is so slow that *miso* and *shoyu* require several

15 months to be produced by fermentation, and the digestion of sludge takes at least 10 days to be completed. Use of enzymes with a view to enhancing the activity of microorganisms and the creation of more active microorganisms by the recombinant DNA technology have been proposed, but these methods have a limited scope of applications and require sophisticated skill.

20 The primary object of the present invention is to provide a method that is capable of enhancing the activity of microorganisms in a far simpler manner than in the conventional methods.

25 This object of the present invention can be achieved by applying an electric current through a liquid culture containing microorganisms.

30 *Figure 1* is a graph showing the relation between the digestion time and the amount of gases produced;

35 *Figure 2* is a graph showing the relation between the cultivation time and the amount of lactic acid produced;

40 *Figure 3* is a graph showing the relation between the cultivation time and the cell mass; and

45 *Figure 4* is a graph showing the time-dependent change of the concentration of dissolved oxygen.

50 The method of the present invention is applicable to a liquid culture containing aerobic microorganisms, as well as to a liquid culture containing anaerobic microorganisms, with the latter being preferred. Preferred liquid cultures include fermentation cultures used in the production of *miso* and *shoyu*, alcohol fermentation cultures, lactic acid fermentation cultures, digested sludge, and activated sludge. Lactic acid fermentation cultures, alcohol fermentation cultures and sludge digestion liquors are particularly preferred.

55 According to the method of the present invention, an electric current is applied through the liquid cultures listed above. The

application of electric current may be performed in an activating tank which has electrodes. The liquid culture in the tank is drawn from the fermenter. After an electric stimulus is applied to the culture in the activating tank, the culture is returned to the fermenter. Alternatively, the application of an electric stimulus may be performed within the fermenter which has built-in electrodes. The specific conditions for current application depends on the type of the liquid culture to be treated: a.c. current or d.c. current may be applied continuously, intermittently or in a pulsive form. The application of an a.c. current in a pulsive form is preferred. The amount of current to be applied ranges generally from 1 mA to 100 A, preferably from 0.01 A to 20 A. If the liquid culture to be treated contains anaerobic microorganisms, application of 0.05 A to 5A of current is preferred. The voltage to be applied is not critical, but it is usually selected from the range of 1 to  $10^3$  volts.

55 The theory behind the method of the present invention is not fully understood. For the purpose of the present invention, the current applied should not be great enough to oxidize or reduce the inorganic or organic matter in the liquid culture to be treated. If d.c. current is applied, water electrolysis unavoidably occurs. However, the amounts of oxygen and hydrogen evolving as a result of electrolysis are negligible. It is generally understood that the application of an electric current to living organisms suppresses the reactions caused by such organisms, and they may die if they are given an excess current. However, according to the present invention, the activity of microorganisms can be significantly enhanced, rather than suppressed, by properly selecting the conditions for current application. Bioreaction consists of assimilation typified by the growth of a microorganism and dissimilation that contributes to the production of metabolites. It is believed that the method of the present invention mainly contributes to enhanced dissimilation. According to the present invention, micro-organisms in a liquid culture are stimulated and their activity enhanced by applying an electric current through the liquid culture.

**Example 1**

A synthetic sample composed of cow milk and starch was digested under anaerobic conditions in a digester having an effective capacity of 2.5 liters. The conditions for digestion were as follows: temperature of digester tank: 37°C; period of digestion: 20 days; organic load: 1.0–1.5 g VSS/1,000 ml-day. The seed sludge was digested sewage sludge. The digested sludge was drawn from the digester at a flow rate of 2,000 ml/min, fed into the activating tank for treatment with an electric stimulus, and returned to the digester. The activating tank contained carbon anode and

cathode, across which an a.c. voltage of 45 volts was applied to cause 2 A of an electric current to flow through the digested sludge. The current was caused to flow for a period of 5 0.03 second per cc of the digested sludge within a prescribed duration of 30 minutes. This application of current was continued intermittently for 8 hours a day. The same experiment was conducted with a comparative 10 synthetic sample of cow milk and starch, except that no current was applied through it during the treatment.

The time-dependent change in the amounts of gases produced is illustrated in Fig. 1, from 15 which one can see that the method of the present invention produced 40–80% more gases than did the conventional method. The gases produced in the method of the present invention contained 45–54% of methane, 20 whereas those produced in the conventional method contained less methane (40–48%). According to the present invention, a methane concentration in the gas produced is increased.

#### 25 Example 2

The effectiveness of the method of the present invention in lactic acid fermentation was checked with an apparatus which was the 30 same as used in Example 1. A liquid culture comprising GAM bouillon (10% glucose) plus *Lactobacillus delbrueckii* was charged into the fermenter and fermented batchwise for 4 days at a controlled temperature of 45°C. During 35 the cultivation period, the culture was drawn out of the fermenter at a flow rate of 2.6 liters/min, fed into an activating tank for treatment with an electric stimulus, and returned to the fermenter. An a.c. voltage of 20 40 volts was applied across the two electrodes in the activating tank, and a current of 2 A was passed through the culture for a period of 0.02 second per cc of the culture in a prescribed duration of 30 minutes. The period of 45 treatment with electric stimulus consisted of three stages: the first 42 hours following the start of the experiment, 54–72 hours, and 87–96 hours. The results of the experiment are shown in Figs. 2 and 3. As is clear from 50 Fig. 2, the method of the present invention produced 30% more lactic acid than was produced in the conventional method. Fig. 3 shows that no *Lactobacillus* was killed by the treatment with an electric stimulus that was 55 given according to the method of the present invention.

#### Example 3

Activated sludge (500 ml, total solids content: 8,000 ppm) was aerated to saturate the dissolved oxygen, and given an electric stimulus by applying an a.c. current of 1.85 A at 100 V for 120 seconds. The treated sludge was mixed with 20 ml of synthetic sewage 60 (BOD: 1,600 mg/1,000 ml), and the mixture 65

was stirred under an air-tight condition to check for the time-dependent change of the concentration of dissolved oxygen. The same experiment was conducted with a mixture of 70 synthetic sewage (20 ml) and another 500 ml of activated sludge that had not been given an electric stimulus. The results of the two experiments are shown in Fig. 4, wherein the inclination of each curve indicates the breathing rate of the activated sludge. The activated sludge that was treated with an electric stimulus according to the method of the present invention breathed at a rate of 60 mg O<sub>2</sub>/1,000 ml-hr, whereas the activated 75 sludge that was given no electric stimulus breathed at 32 mg O<sub>2</sub>/1,000 ml-hr. In other words, the activated sludge given an electric stimulus by the method of the present invention came to breathe 53% faster than the 80 sludge treated by the conventional method. It is therefore concluded that the present invention is also effective in enhancing the activity of aerobic microorganisms.

#### 90 CLAIMS

1. A method of promoting the bioreaction of microorganisms in a liquid culture by treating said culture with an electric stimulus.
2. A method according to Claim 1 wherein said electric stimulus is imparted in the form of a pulsive current.
3. A method according to Claim 2 wherein said pulsive current is an a.c. current.
4. A method according to any one of the preceding claims wherein 0.01 A to 20 A of an electric current is applied.
5. A method according to Claim 4 wherein the liquid culture is the digested sludge.

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